Genome-scale modeling of *Synechocystis* metabolism

A status report

Timo Maarleveld, Frank Bruggeman, Bas Teusink

Frank J Bruggeman (frank.bruggeman@sysbio.nl)
Systems Bioinformatics, VU University
Life Science, CWI, Amsterdam

NETHERLANDS INSTITUTE FOR
SYSTEMS BIOLOGY

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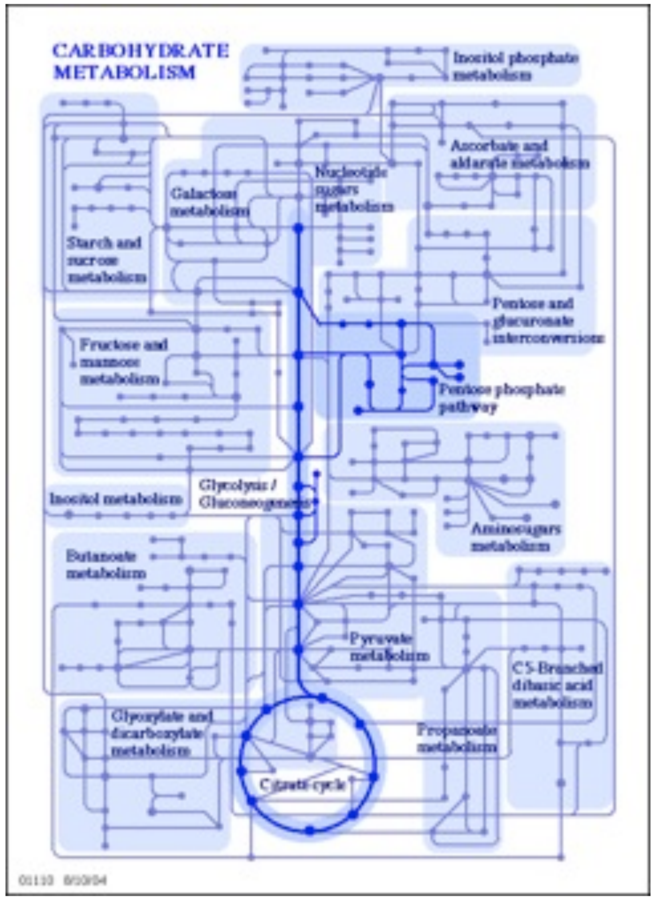


Phases:

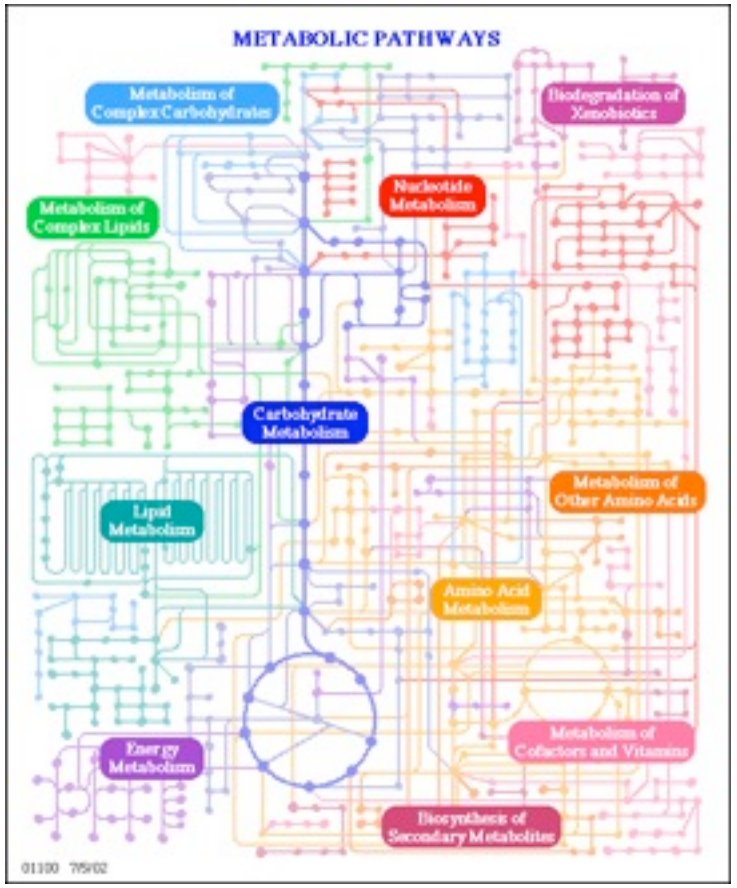
Current status:

1. Stoichiometric model of entire metabolism
2. Circadian clock dependency; day-night rhythm

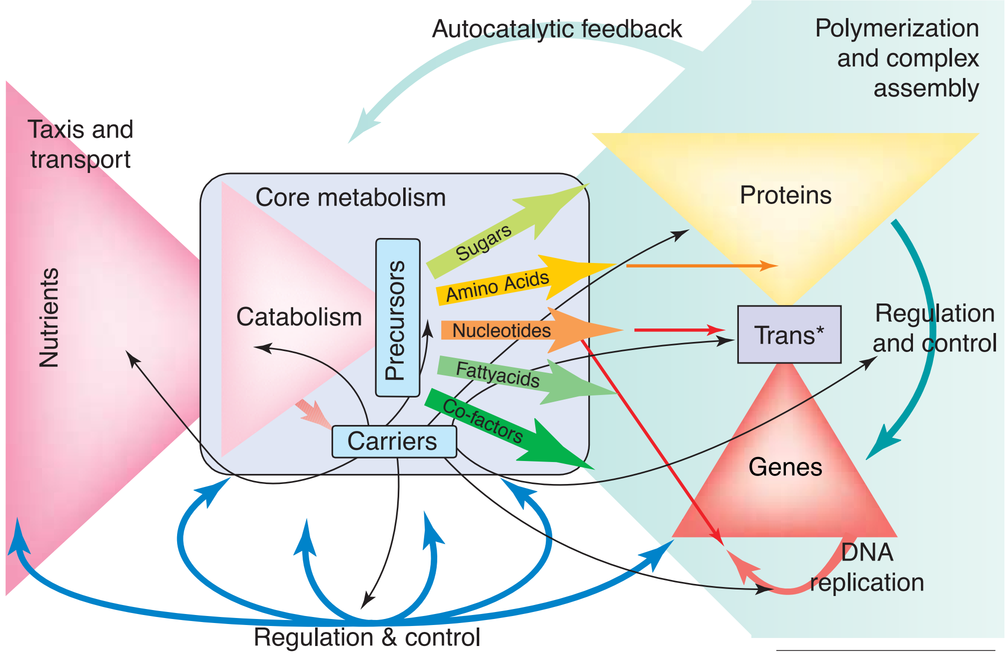
Next:

3. Incorporation of kinetic data
4. Consideration of reactor conditions

A system's perspective on metabolism

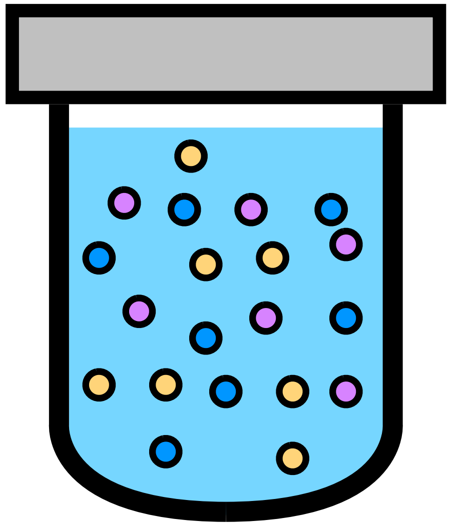


Metabolic network



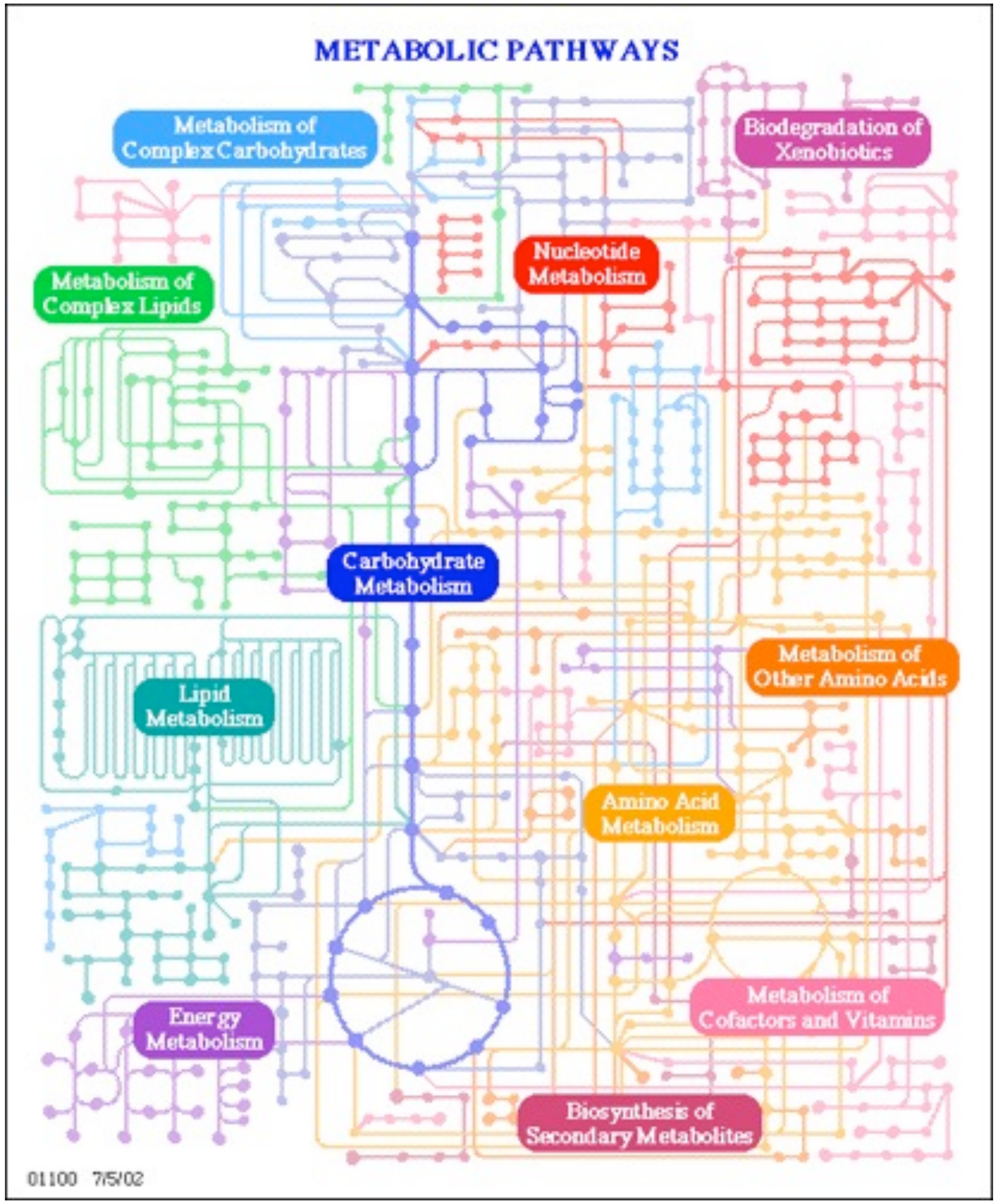
TRENDS in Biotechnology

Growth control



Reactor and environment

GENOME-SCALE METABOLIC NETWORK MODELS



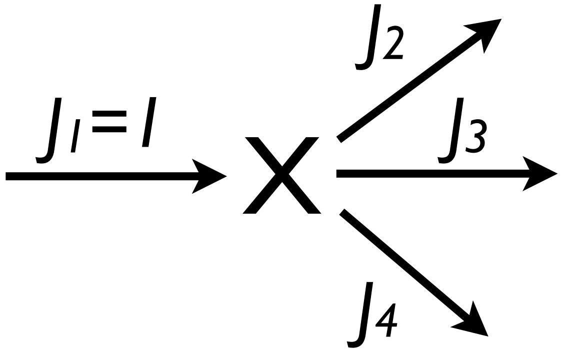
INPUT

✓Entire metabolic network
✓Reaction stoichiometry
✓ Steady state assumption
✓ min/max flux bounds
✓ optimization approach
✓ NO KINETICS

OUTPUT

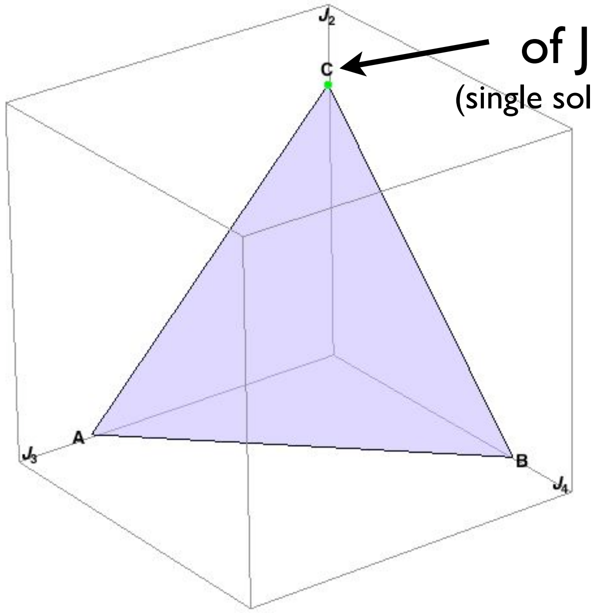
➡ Well-defined mathematical problem/linear programming
➡ Industrially-relevant predictions can be made
➡ Fundamental questions about metabolic network organization and whole cell physiology can be asked

Flux balance analysis in a nutshell
(our main modeling formalism)



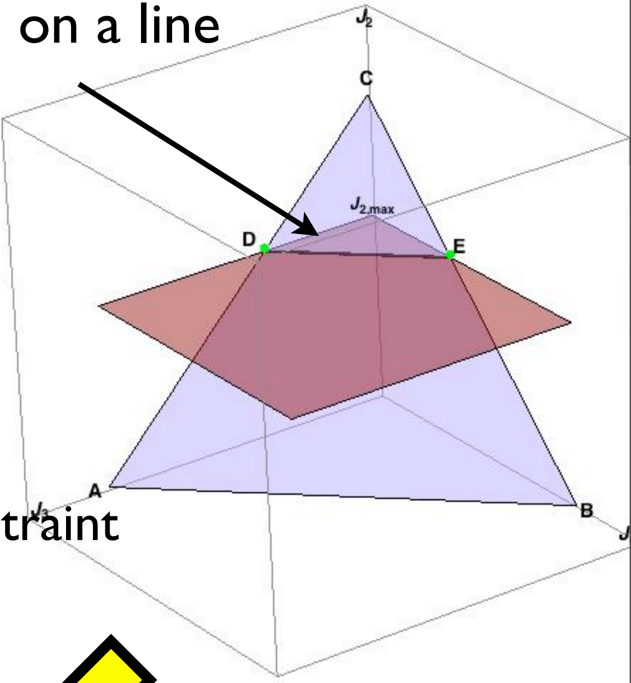
Solution flux space constrained by:
- steady-state
- stoichiometry
- all fluxes positive (irr)
- input flux

Objective: max J_2



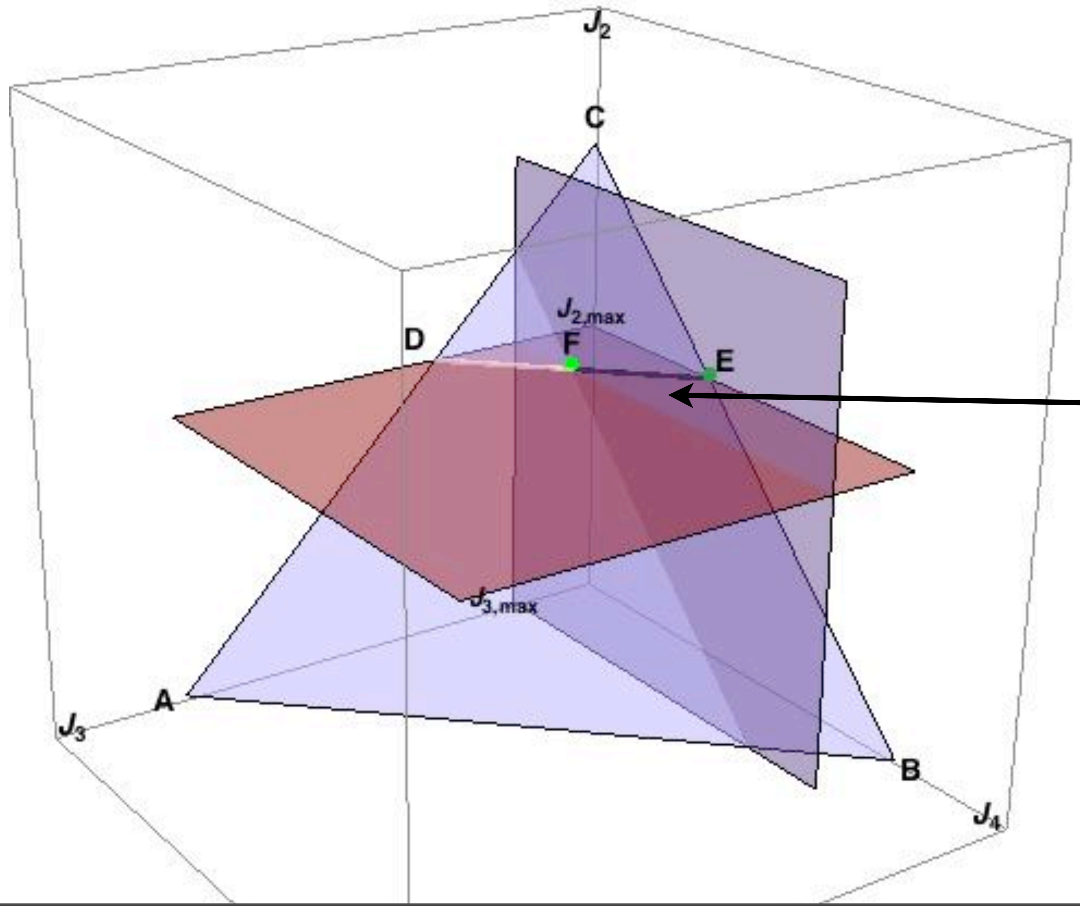
maximal value of J_2
(single solution)

+ capacity constraint
$J_2 \leq 0.6$



All solutions to maximal values of J_2 lie on a line

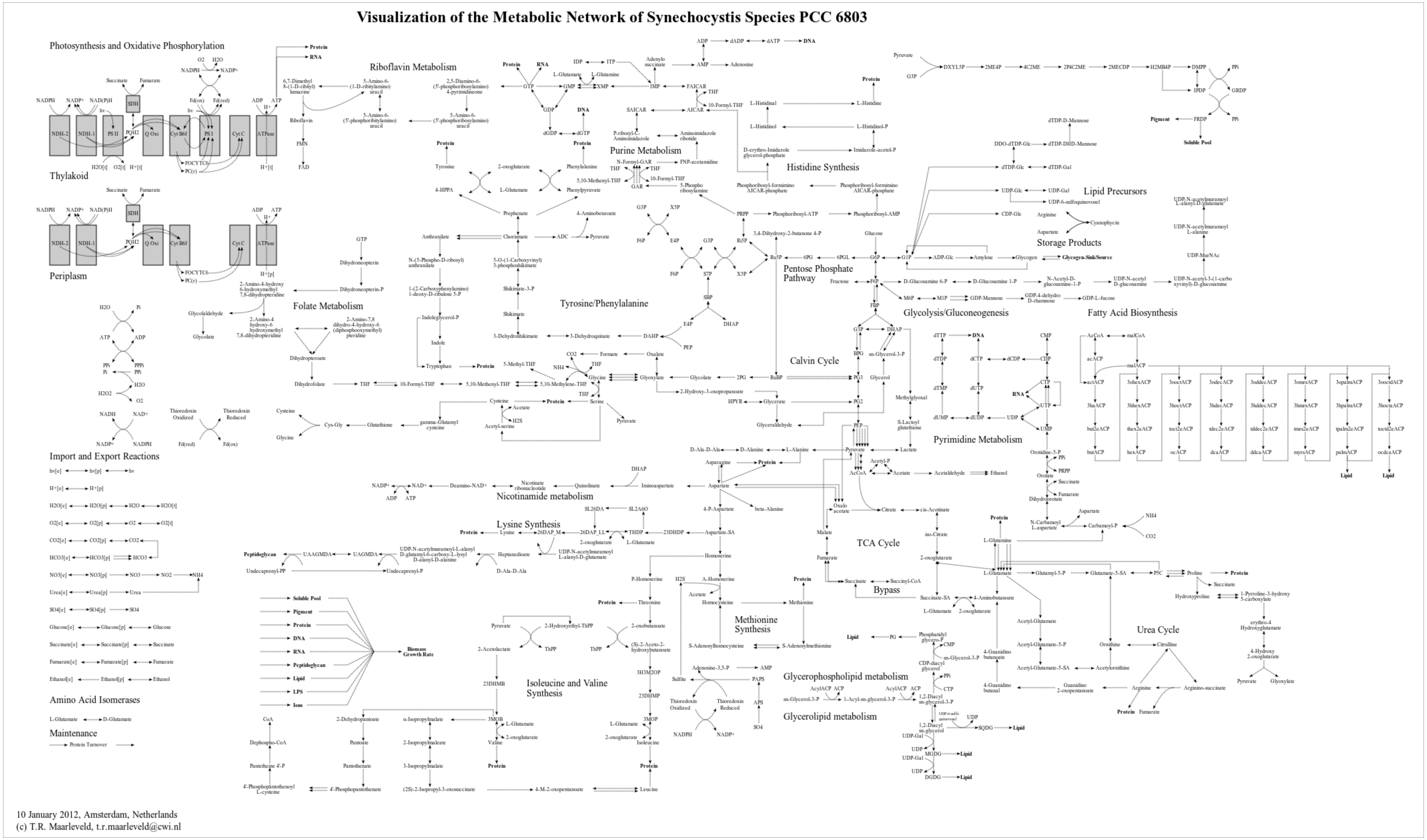
+ capacity constraint
$J_3 \leq 0.2$



All solutions to maximal values of J_2 lie on a line

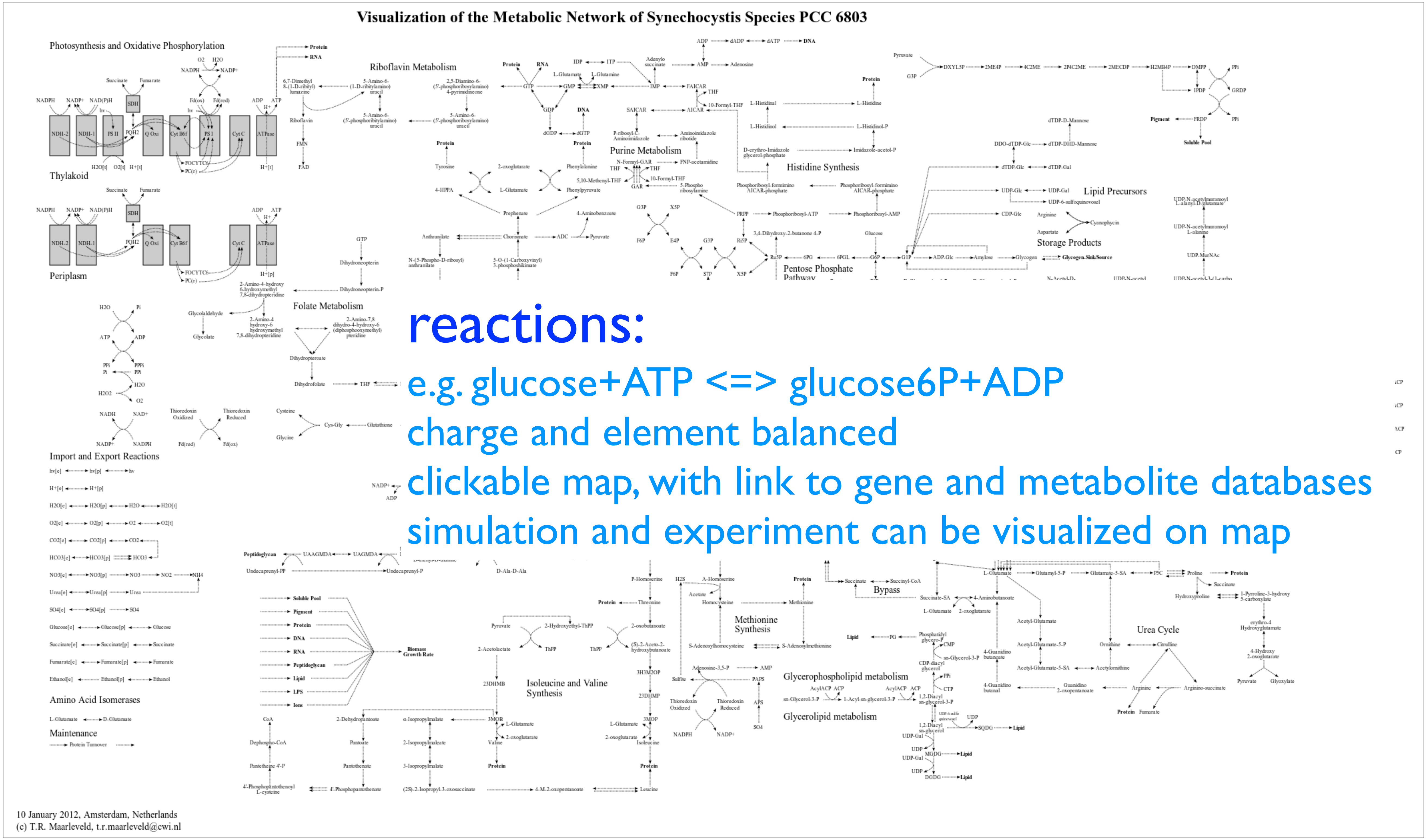
An whole space of optimal flux solutions can exist

Model status



Genome-scale model of *Nogales et al. 2012* expanded with reactions from *Knoop et al. 2010*
906 reactions and 818 metabolites

Model status



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reactions:

e.g. glucose+ATP <=> glucose6P+ADP

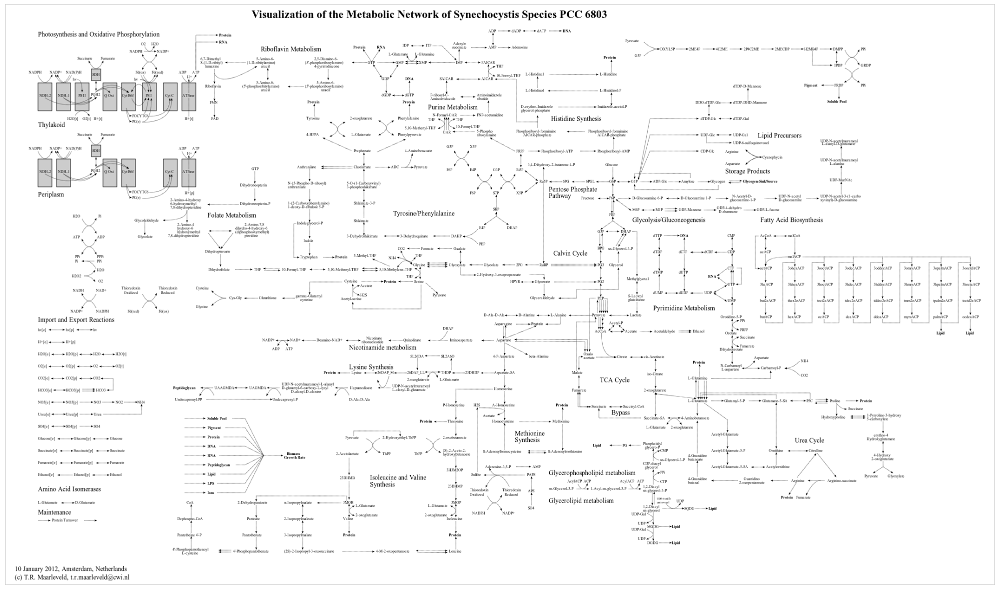
charge and element balanced

clickable map, with link to gene and metabolite databases

simulation and experiment can be visualized on map

Genome-scale model of *Nogales et al. 2012* expanded with reactions from *Knoop et al. 2010*
906 reactions and 818 metabolites

Simulation procedure



translation into
linear program
(or MILP....)

$$\mathbf{Nv} = \mathbf{0}$$

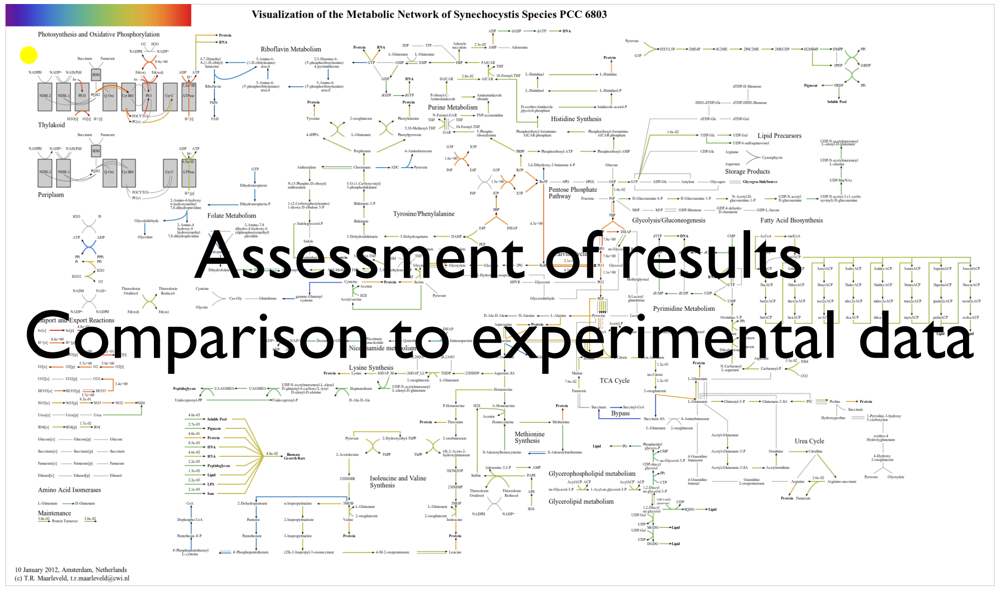
$$\forall i : v_i^{min} \leq v_i \leq v_i^{max}$$

$$\max(v_X = \mu)$$

Computation in Pysces
(in-house simulation package
for dynamic and stoichiometric
modeling of large system)

Output

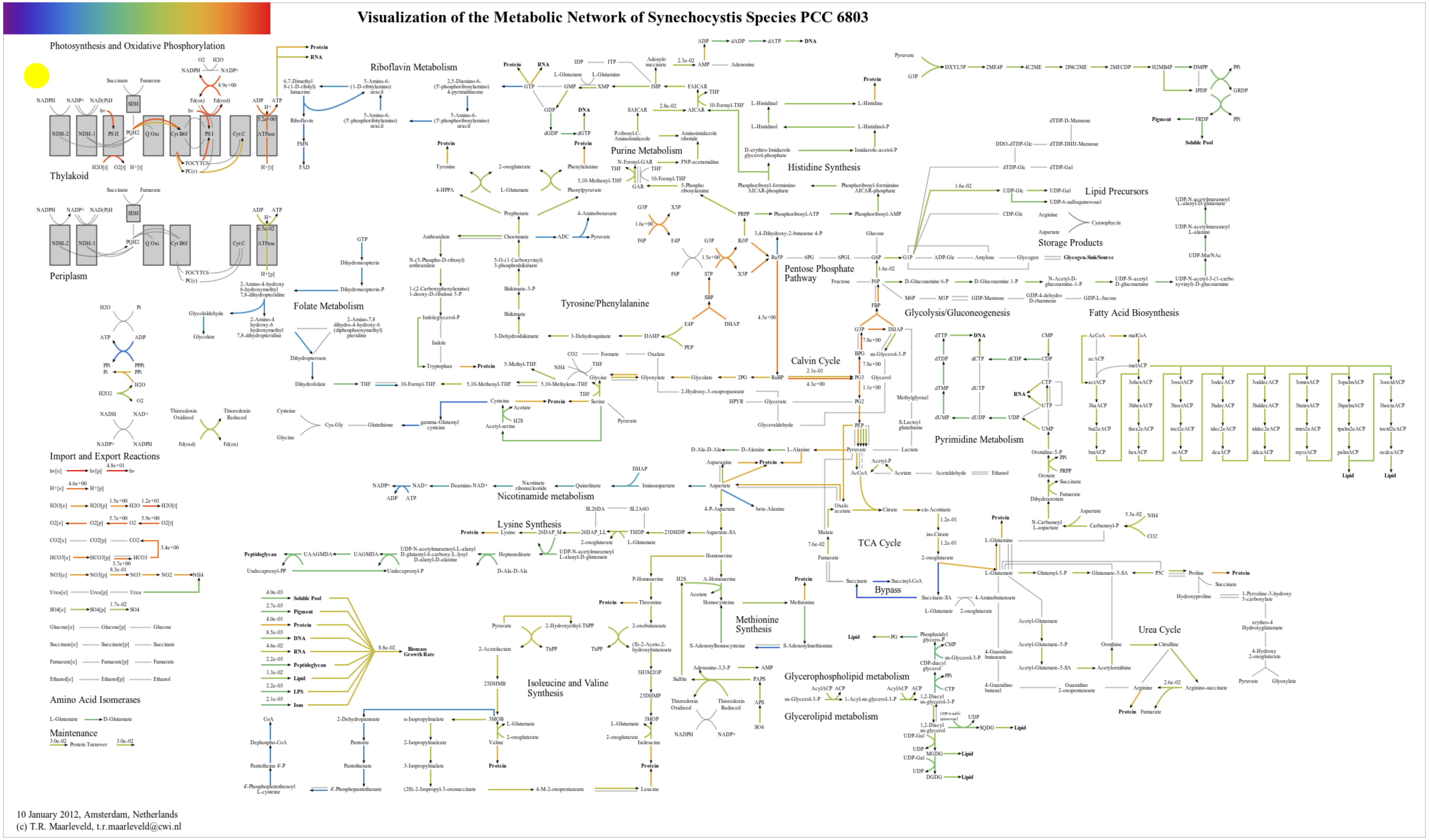
visualization
on network map



Assessment of results
Comparison to experimental data

Consideration of day/night rhythms (1st attempt)

Optimization of growth during the day



Visualization of the Metabolic Network of Synechocystis Species PCC 6803

10 January 2012, Amsterdam, Netherlands
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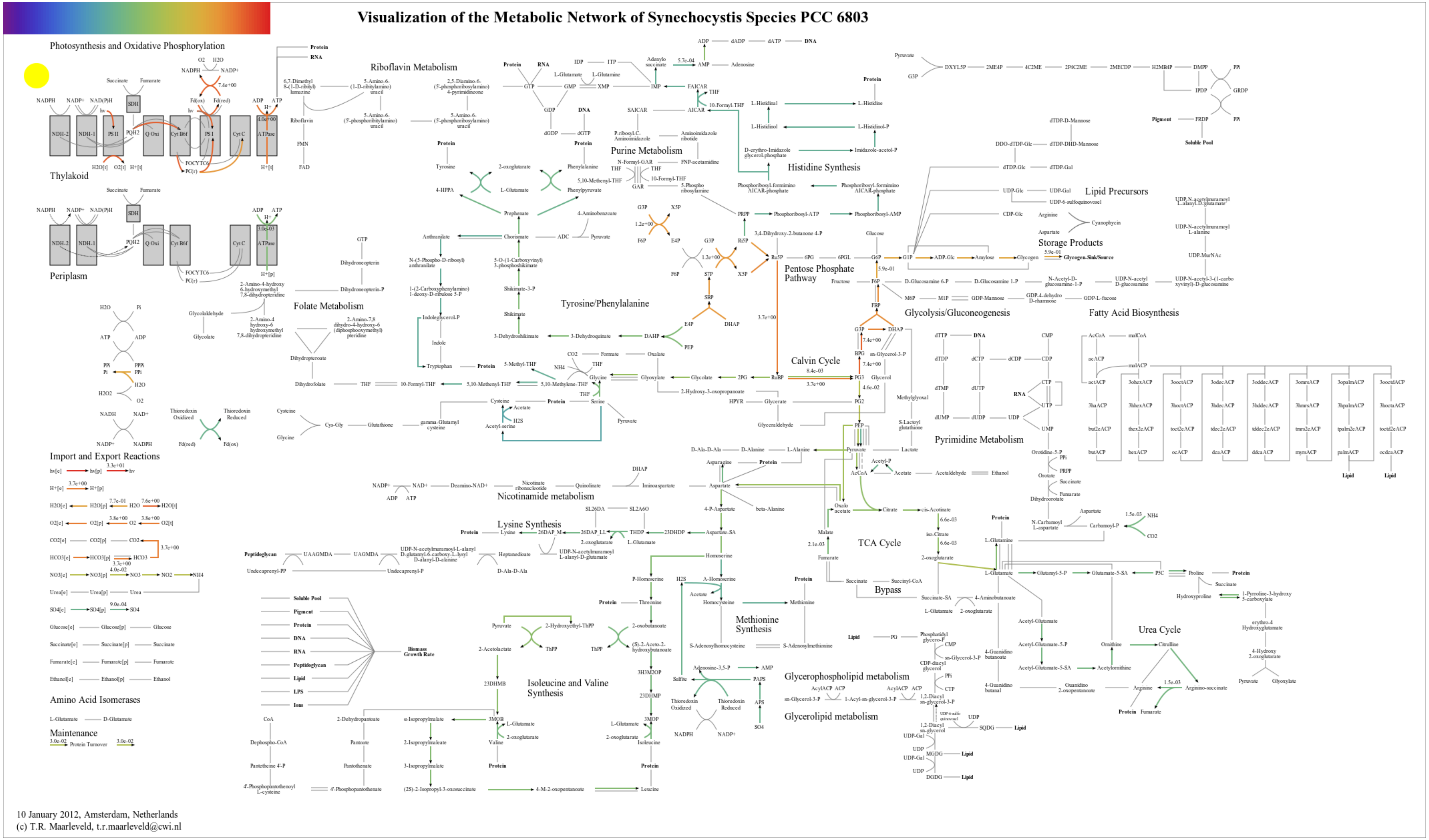
Assumptions:

- biomass yield (g DRW/mol photon) is optimized. Growth rate 0.088 hr^{-1} (exp data).
- protein turnover equals maintenance costs; CO_2 as carbon source

Consideration of day/night rhythms (1st attempt)

Production of glycogen to prepare for the night

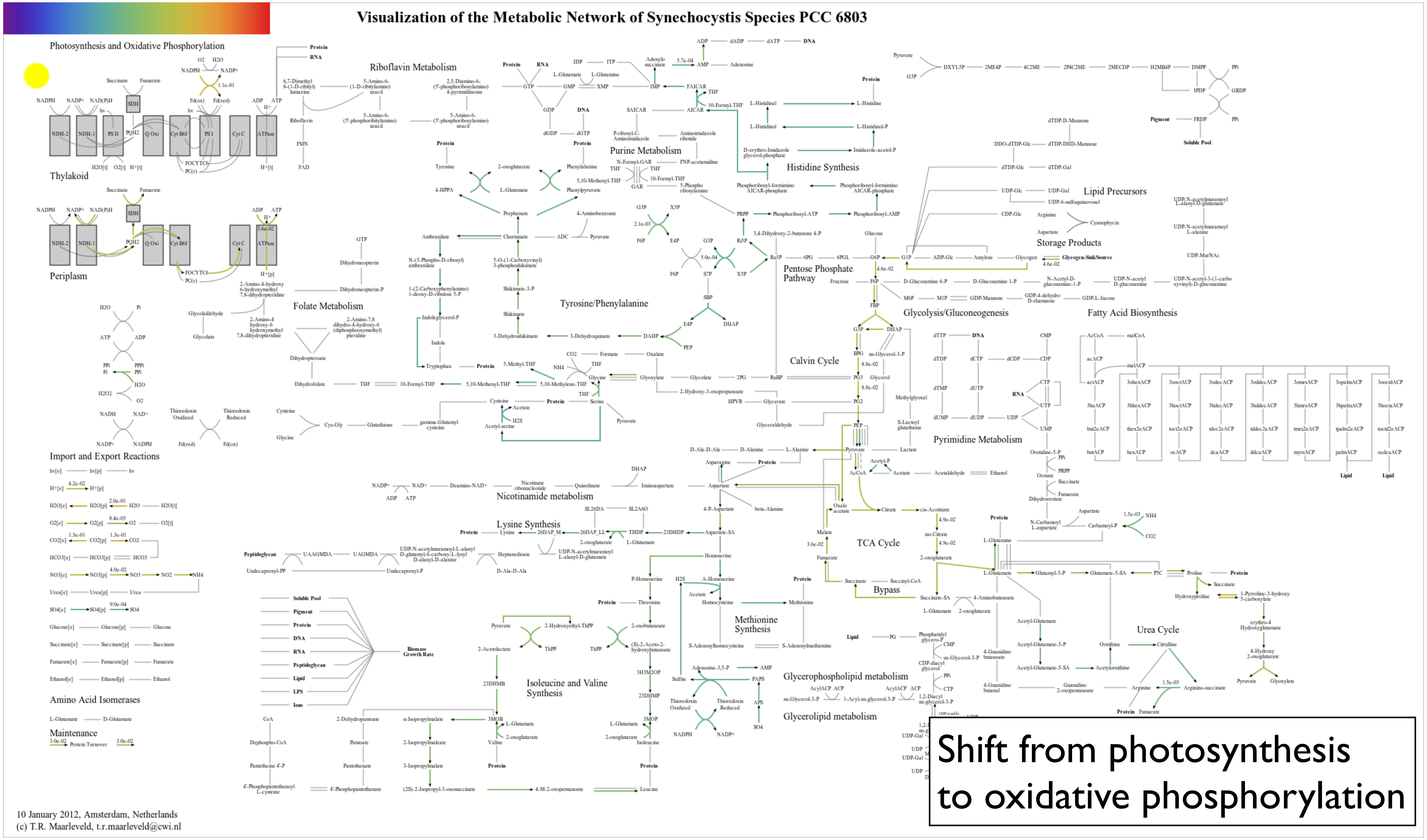


Assumptions:

- glycogen yield (mol glycogen/mol photon) is optimized.
- protein turnover equals maintenance costs; CO_2 as carbon source

Consideration of day/night rhythms (1st attempt)

Maintenance and glycogen consumption during the night

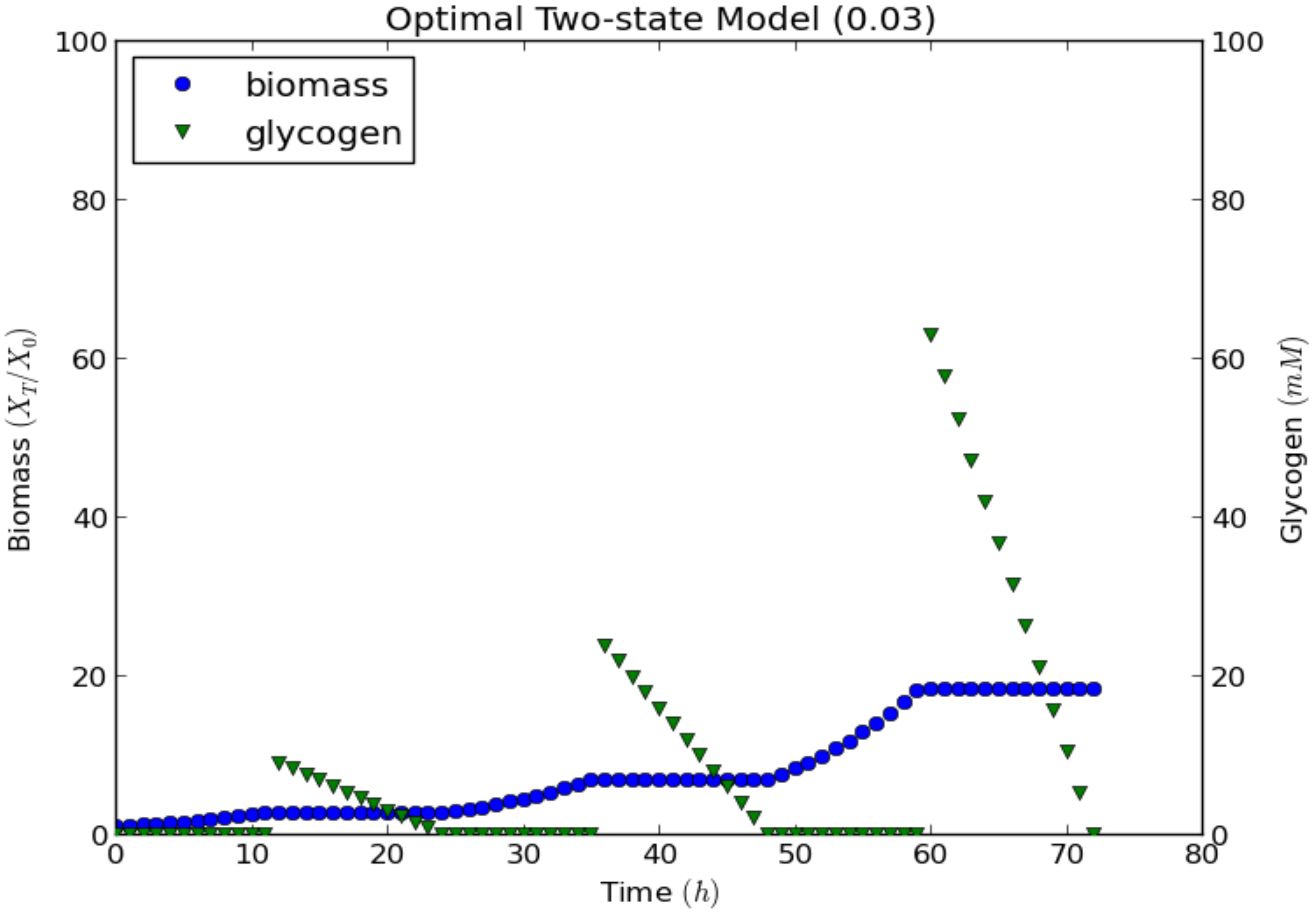


Shift from photosynthesis to oxidative phosphorylation

Assumptions:

- protein yield (mol protein/mol glycogen) is minimized to obtain the "cheapest" maintenance strategy.

Optimal metabolism (growth & glycogenolysis) over time.

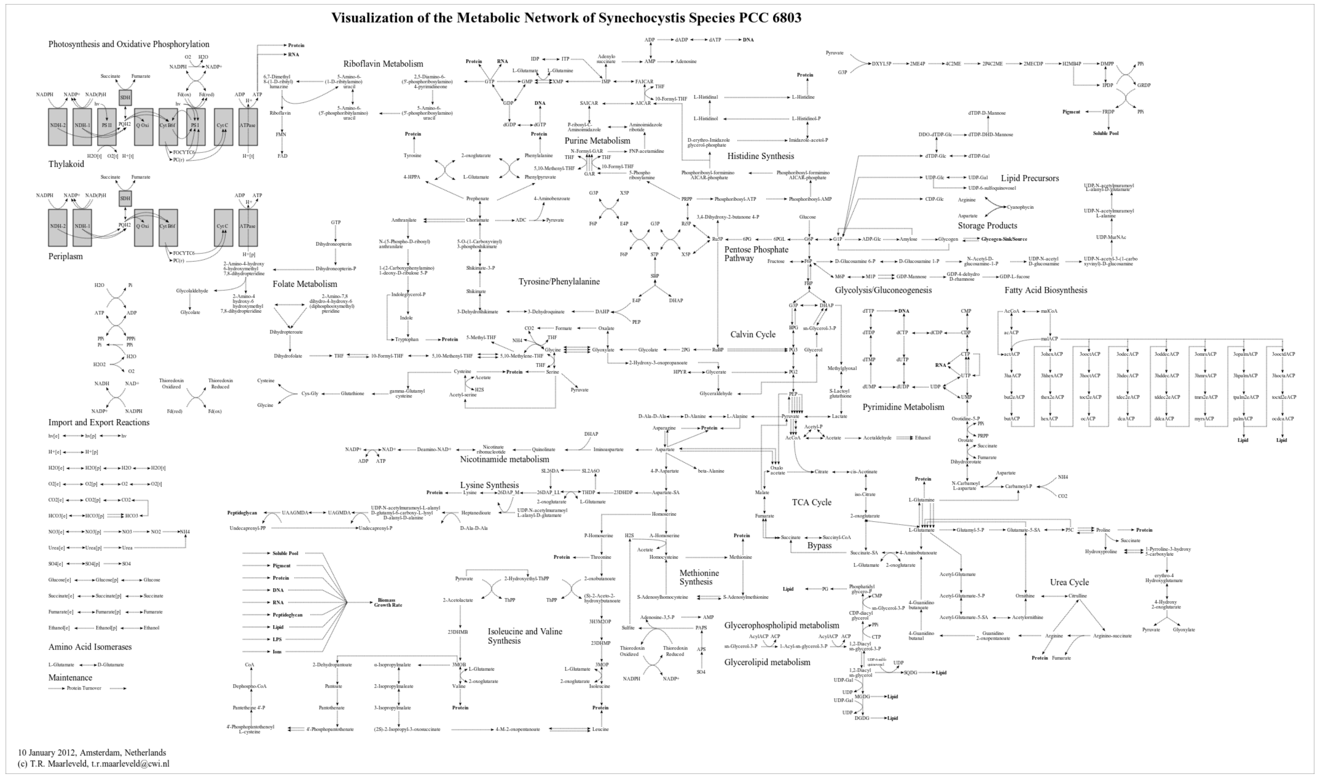


Metabolic strategy optimization: optimization of biomass over circadian cycles

We estimated protein turnover rates, which sets the glycogen requirement for the night phase.

The model predicts:
- exponential growth during the day
- glycogen synthesis just before dark
- no growth during the night

Future plans:
Simulation strategies for metabolic engineering of *Synechocystis*

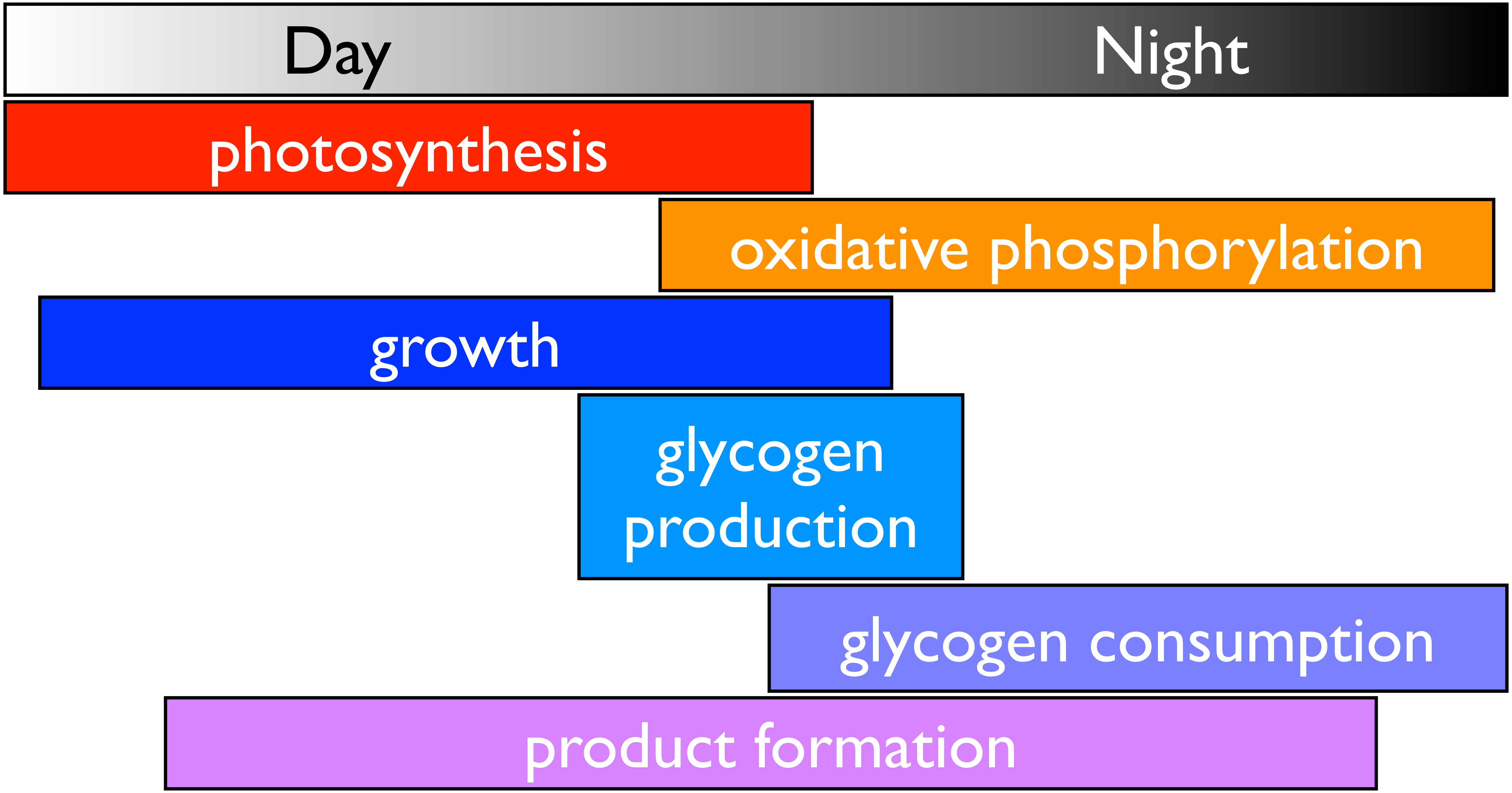


For a circadian-rhythm enforced simulation:

- Assessment of metabolic flexibility
  - identification of flux routes that reduce product yield
- Identification of intracellular metabolic fluxes that limit production flux
  - e.g. assessment of redox problems
- Design of optimal knockout strategy

We have the programming code for classical problems. Proper linkage to circadian rhythm and explicit consideration of cell growth requires further attention. This is a new problem in the genome-scale modeling field. No reason to believe that this cannot be done.

Future plans:
Simulation strategies for metabolic engineering of *Synechocystis*



Day

Night

photosynthesis

oxidative phosphorylation

growth

glycogen production

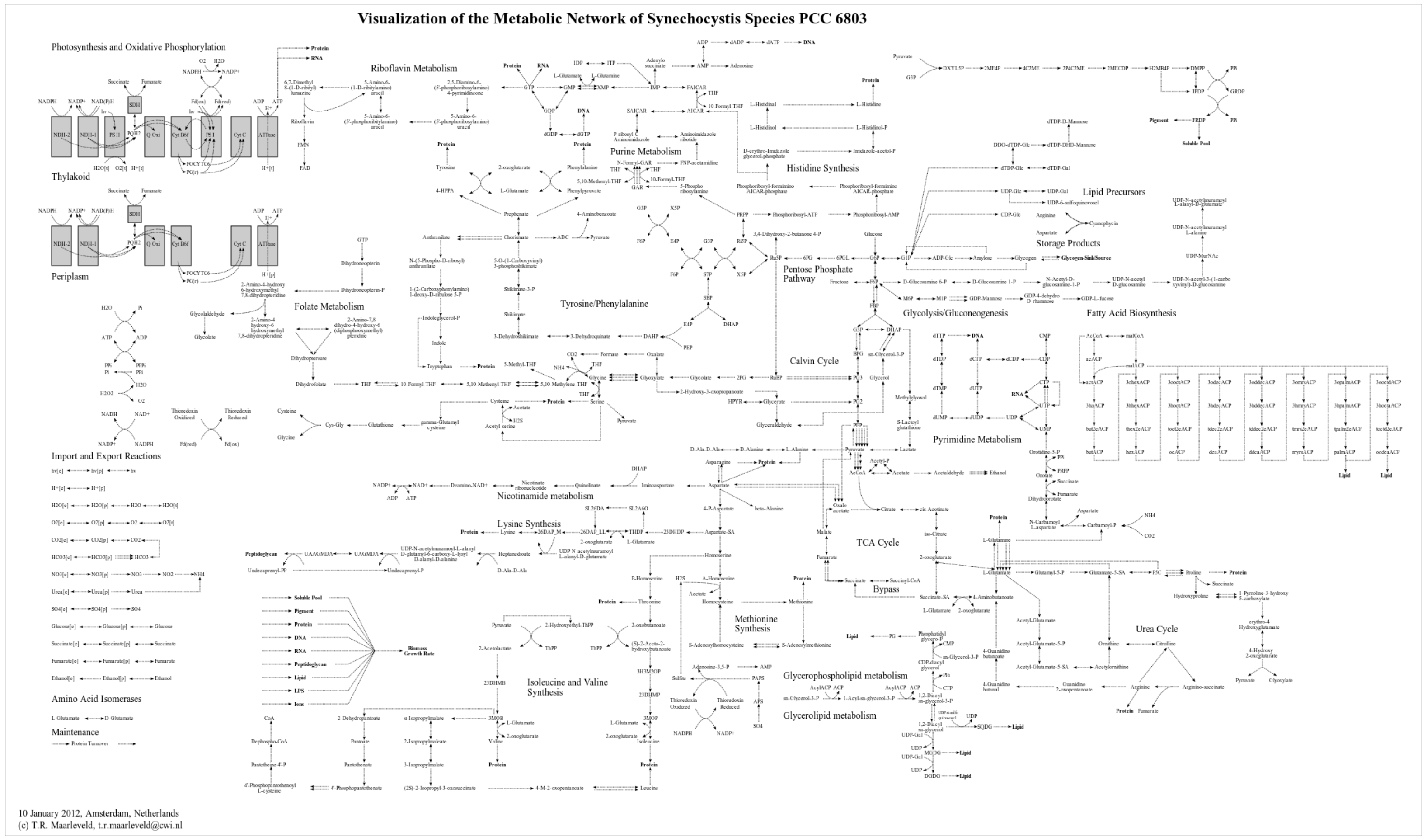
glycogen consumption

product formation

What is unclear is and will be investigated next:

1. when those processes should start and end
2. which metabolic pathways are active and how they change in activity over time
3. comparisons with experimental data (flux, microarrays)

Future plans:
Incorporation of kinetic models to integrate kinetic control

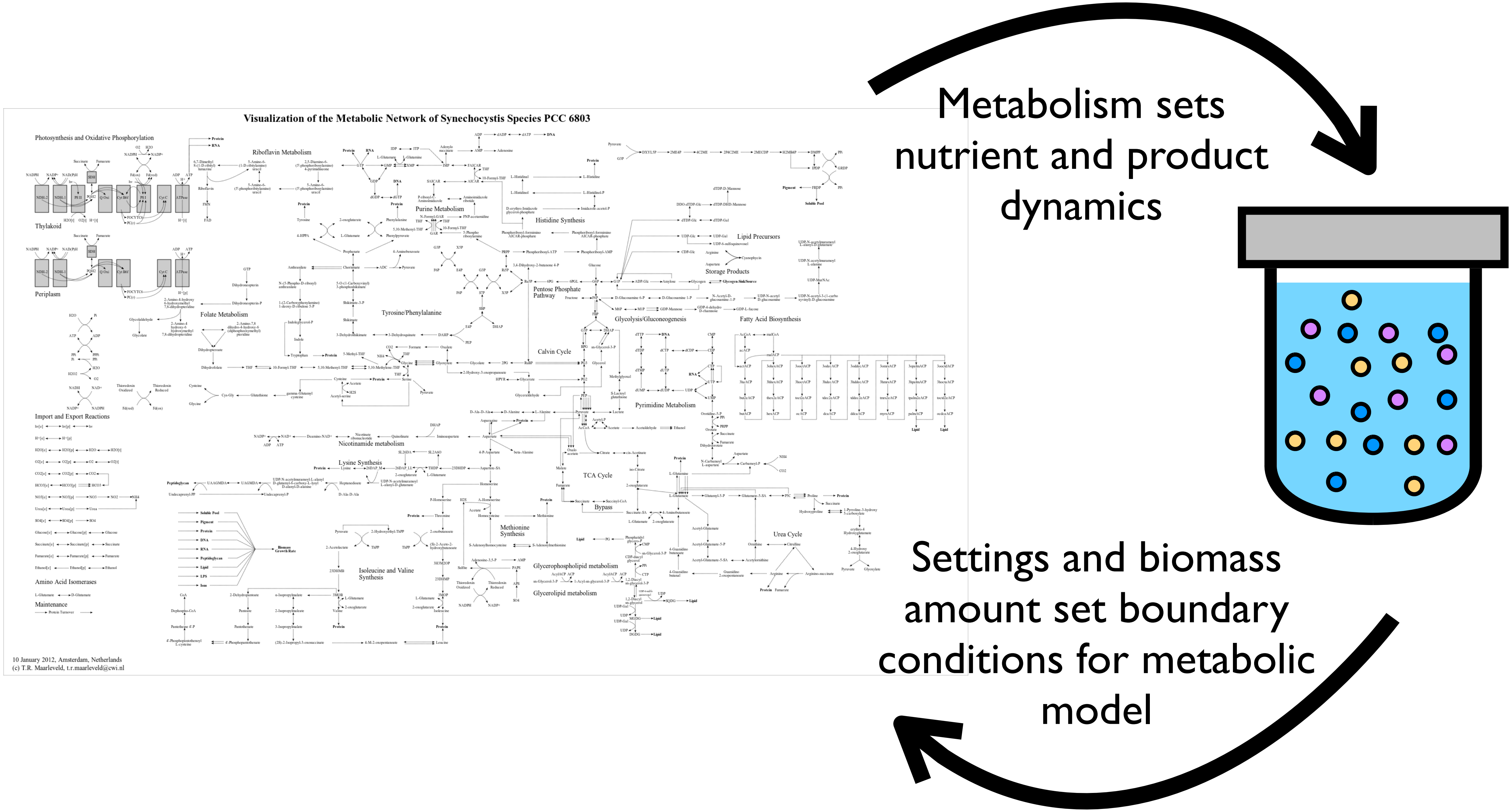


Candidates:

- Calvin cycle (exists)
- Photosynthesis (Grondelle)
- Circadian rhythm (Ten Wolde)
- Glycolysis/glycogenolysis (extension of in-house models)

This leads to a so-called hybrid model (kinetic + stoichiometric segments)

Future plans:
Consideration of reactor conditions



Metabolism sets nutrient and product dynamics

Settings and biomass amount set boundary conditions for metabolic model

This leads to a so-called multi-scale model

Conclusions

- Investigation of the entire metabolism of *Synechocystis*
- Incorporation of day-night rhythms
- Identification of optimal metabolic strategies for growth and product formation

Next,

- incorporation of kinetic models
- consideration of reactor conditions

Thanks to:

Timo Maarleveld & Bas Teusink